PHYSICO-CHEMICAL ASSESSMENT OF LABELED FREEZE DRIED KITS OF TRASTUZUMAB-IMMUNOCONJUGATES SIGNIFICANT FOR BREAST CANCER THERAPY

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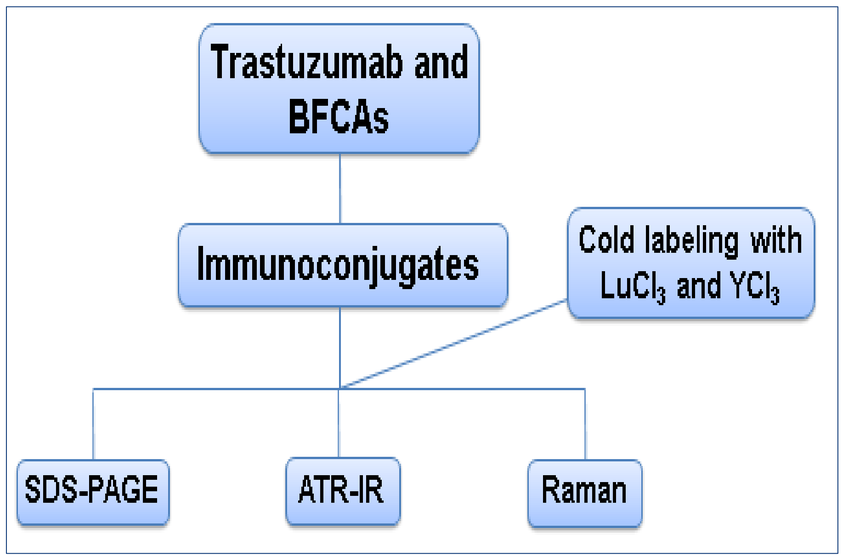
INTRODUCTION

Trastuzumab is a humanized IgG1 monoclonal antibody for treatment of HER2 positive breast cancer. Due to the significant potency in various malignancies and easy detection of radioactivity with outside scintigraphy, radioimmunoconjugates have become a part of many clinical trials. The aim of this study is to formulate a stable lyophilized trastuzumab-immunoconjugates with bifunctional chelators (BFCAs): *p-SCN-Bn-DTPA*, *p-SCN-Bn-DOTA*, *p-SCN-Bn-1B4M-DTPA* and *in vitro* examinations by *reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis* (SDS-PAGE), *attenuated total reflectance-infrared* (ATR-IR) and *Raman spectroscopy.* These techniques provide valuable molecular structure information and are convenient for verification of changes in the secondary structure after antibody manipulation and exposure to stress conditions during the processes of conjugation, lyophilization and labeling.

MATERIAL AND METHODS



RESULTS

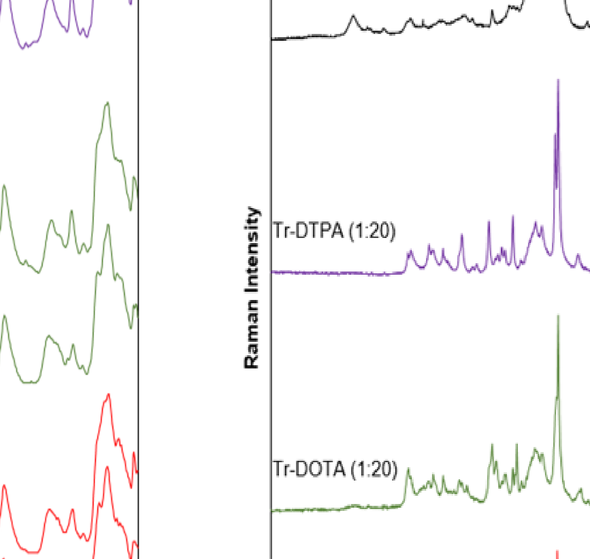


Fig. 1 Reducing SDS-PAGE of:
Trastuzumab (Tr) 1 mg/ml (1);
DTPA-Tr (1:20) (2);
DOTA-Tr (1:20) (3);
1B4M-DTPA-Tr (1:20) (4).

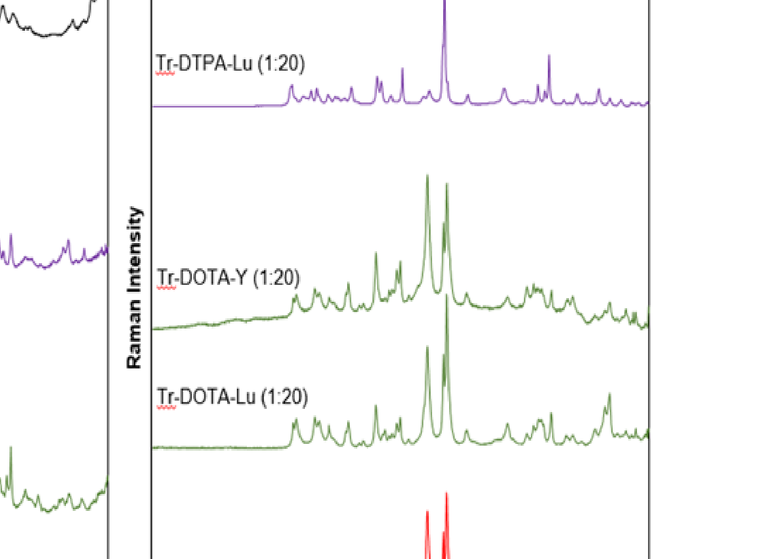


Fig. 2 Reducing SDS-PAGE of:
Y-DTPA-Trastuzumab (1:20) (1);
Lu-DTPA-Trastuzumab (1:20) (2);
Y-DOTA-Trastuzumab (1:20) (3);
Lu-DOTA-Trastuzumab (1:20) (4);
Y-1B4M-DTPA-Trastuzumab (1:20) (5);
Lu-1B4M-DTPA-Trastuzumab (1:20) (6).

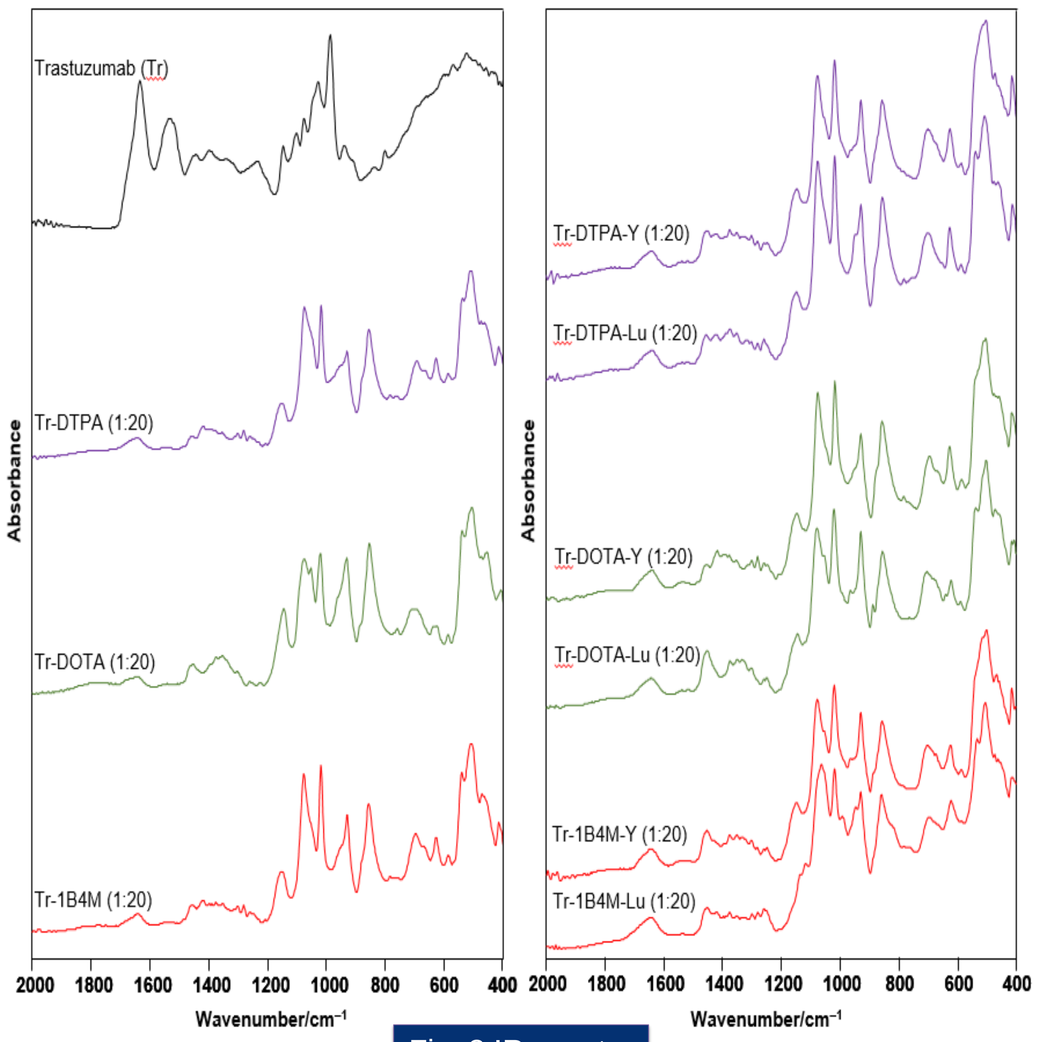


Fig. 3 IR spectra

Fig. 4 Raman spectra

Characteristic Raman bands

S-S	Tyr	Trp	Phe	Indol ring	Amid band I	Amide band III
400-700 cm^{-1}	647cm^{-1}, 760-790 cm^{-1}	757 cm^{-1}, 878 cm^{-1}, 1337 cm^{-1}	1004-1060 cm^{-1}, 1610 cm^{-1}	1560 cm^{-1}	1668-1688 cm^{-1}	1235-1260 cm^{-1}

Characteristic IR bands

Amide band I	Amide band II	Amide band III	Amide band IV and V	Amid band VI
1640-1645 cm^{-1}	1480-1575 cm^{-1}	1233-1300 cm^{-1}	620-810 cm^{-1}	500-595 cm^{-1}

Table. 1 Characetristic Raman andIR bands

CONCLUSION

The promising results from electrophoresis and vibrational spectroscopy are good basis for further radiolabeling of immunoconjugates with ^{177}Lu and ^{90}Y for treatment and imaging of HER2 positive lesions.